National Wildlife Health Center, Madison, Wisconsin 2008 protocol for collection, storage, and shipment of separate oral-pharyngeal (OP) and cloacal (CL) swab samples. Primary use of this protocol will be in Alaska; elsewhere when collecting separate swabs. Please coordinate shipments with Richard Zane (608-270-2481) or Diane Goldberg (608-270-2455)

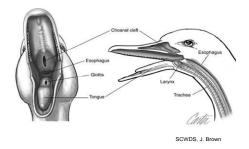
Background Information

Oral-pharyngeal (OP) and cloacal (CL) samples will be collected from each bird. The vials are paired and prelabeled with USGS case and accession numbers, and marked with OP for the oral-pharyngeal swab, and CL for the cloacal swab. Caps of vials for OP swabs are clear; caps of vials for CL swabs will be colored. Vials will be shipped on dry ice and can be stored at standard freezer temp (-20°C) for the duration of the field season. Once thawed, virus transport media is good for 7 days refrigerated at 4°C. Unused media vials may be refrozen twice. As samples are collected, keep vials out of sunlight in a cooler on ice or blue ice packs during the day's work. After returning from the field, transfer vials to liquid nitrogen vapor shippers (alternatives: store in ultra-low freezers at -80°C or double-bagged on dry ice) but do not freeze at standard freezer temperature (-20°C). Alternatively, samples can be kept refrigerated or on frozen ice packs and shipped to NWHC if they will arrive at NWHC within a **maximum** of 72 hours of collection.

Oral-pharyngeal (uncolored vial cap)

- 1. Thaw appropriate number of paired vials of media at refrigerator temperature (4°C) or on ice and keep chilled with wet/blue ice packs in a cooler during the day of collection.
- 2. Check the bird for band number(s) and write the 4-letter species code and band number on the paired vial with fine-tip Sharpie.
- 3. Unwrap a Dacron swab from the stem-end of the packaging (store swabs so they do not get wet). Open oral cavity (technique will depend on species and whether alive or dead; take care to avoid injury with live birds), insert swab, and with gentle front to back motion swab along both sides of the base of the tongue. Continue toward the back of the oral cavity and swab the area behind the tongue, avoiding the glottis (opening to the trachea). Remove the swab from the oral cavity by bringing it forward in contact with the roof of the mouth; swab over (not in) the choanal slit along the top (dorsal side) of the oral cavity.





4. Insert the swab into the paired vial with the uncolored top (labeled OP) and swirl against the inside of the vial. Lift the swab about 1/2" from the bottom of the vial and bend the stem over the edge of the vial to break the stem off, or cut the stem with scissors or a wire cutter, so that the swab remains in the vial and the cap can still be screwed on tightly.

5. The end of the swab and a portion of the stem should be left in the tube. Wipe the scissors or wire cutters with alcohol between each use.

Cloacal swabs (colored vial cap)

- 1. Unwrap another Dacron swab from the stem-end of the packaging, remove swab and insert the entire head of the swab into the cloaca. Appling gentle pressure, rotate the swab inside the cloaca two or three times.
- 2. Shake off large pieces of feces and insert the swab into the paired vial with the colored top and labeled CL, following steps #4 & 5 above.
- 3. Keep tubes on ice or ice packs and out of direct sunlight in a cooler for transport to camp. Transfer vials to nitrogen dry shipper as soon as possible (or other storage in Background, above)



Field data to send to NWHC:

Use the National HPAI Early Detection System through the Wildlife Disease Information Node

Visit http://wildlifedisease.nbii.gov/ai and download appropriate Excel worksheet Cross reference band or field number with NWHC case and accession number